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In Vitro Callus Induction Studies of *Physalis minima* (L). An Important Medicinal Plant

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Abstract
The present study deals with the *In Vitro* callus induction of *Physalis minima* L. is an important medicinal plant which belongs to the family Solanaceae. It is widely used for inflammation, enlargement of the spleen and abdominal troubles**.** The efficient *in vitro callus* induction of *Physalis minima* was achieved from internode and leaf explants on MS medium with B5 vitamins and different concentrations and combinations of BAP, IAA, NAA and 2.4-D.The highest rate of callus induction was observed from the indrtnode and leaf explants on MS medium with BAP (3.0 mg/l) + NAA (1.0 mg/l) + 2.4-D (1.0 mg/l) and IAA (2.0 mg/l).The regenerated callus was transferred to half strength MS medium fortified with IAA (2.0 mg/l The Callus induction was successfully the current study showed efficient *in vitro* callus induction capabilities of *Physalis minima* L.

Keywords: Callus induction; MS medium; BAP and 2.4-D.

Introduction

Physalis minima L. belongs to the family Solanaceae, which is commonly known as wild Cape gooseberry in English.kupanti, Budda and Budamma in Telugu. It is a small delicate, erect, annual, pubescent herb. Leaves are ovate to cordate, pubescent, delicate, estipulate acuminate having reticulate palmate venation. Flowers are pedicellate, hermaphrodite; complete, solitary, fruits are berry. The plant is reported as diuretic, laxative, useful in inflammations, supplement for Vitamin c, enlargement of spleen and abdominal troubles (Koster. *et al*, 1959) [7]. The *Physalis minima* is an annual herbaceous plant commonly found in tropical countries like India, Africa, Afghanistan, Srilanka and is an Ayurvedic plant with medicinal importance. (Joshi anand *et al*, 2014) [8]. Medicinal plants have at least one of their parts leaves, stem, barks or roots used for therapeutic purposes (Bruneton, 1993) [2]. Recently, medicinal plants have become important for the treatment of different disease conditions, such as diabetes, malaria, anaemia (Fola, 1993) [3]. The availability and relatively cheaper cost of medicinal plants, makes them more attractive as therapeutic agents when compared to modern medicines (Agbor and Ngogang, 2005; Agbor *et al*., 2005) [4, 6].

Materials and Methods

Plant Material

Physalis minima L. plants were collected from Vayalur, Tiruchirappalli district, Tamilnadu, India and were successfully planted in National College herbal garden for further use. The plants were maintained in the Department of Botany, National College. For the initial experiments, healthy internodal and leaf explants were collected from two months old plant.

Selection and Surface Sterilization of Explants

A selection of internodal segment and leaf explants for our experimentation, we have chosen them for further studies on the effect of growth hormones like Benzyl Amino Purine (BAP), Naphthalene Acetic Acid (NAA) and 2,4-Dichlorophenoxyacetic acid (2, 4-D). All explants were first washed under running tap water for 30 min and then washed with 2-3 drops of Tween 20 detergent solution for 15-20 min. Traces of Tween 20 solution were removed by washing 6-7 times with distilled water and transferred to Laminar air flow chamber. The explants were surface sterilized with 0.1 % (w/v) $HgCl_2$ solution for 4 min, and then washed with sterilized distilled water. Then 70%ethanol was added for 5 min and then explants were washed with sterilized distilled water for 5-7 times the explants were then cut to the required size and inoculated onto culture medium. All the explants were placed horizontally on the medium, and the leaves were placed with their dorsal side in contact with the medium.

Culture Medium and Conditions

The culture medium used for the explants was MS medium (Murashige and Skoog, 1962) with B5vitamins (Gamborg *et al*., 1968) [1] supplemented with 3% (w/v) sucrose and pH was adjusted to 5.8 with 1N NaOH or HCl before addition of 0.8% (w/v) agar (Hi media, India) and enriched with varying concentrations of BAP and BAP in combination with NAA, 2, 4-D, IAA to determine the optimum growth regulator levels. The following concentrations tested for BAP (1.0-3.0 mg/l), IAA (0.5-1.5 mg/l), 2, 4- D (0.5 mg/l) NAA (0.5-1.0mg/l). Molten media were dispensed into test tubes (Borosil, India) (25×150mm; 10ml) and closed with non-absorbent cotton plugs and media were autoclaved at 104 kpa and 121^0C for 20 min. The cultures were maintained at 25± 2^0C under a 16 hour photoperiod of 35µ molm-2 s-1 irradiance provided by cool white fluorescent light with 55-65% relative humidity. For hardening off, 7 to 8 weeks old rooted shootlets were removed from the culture flacks. After the agar with the running water they were transferred into small polythene bags containing sterilized cow dung, sand and red soil (1: 1: 1) and kept in a mist house. After acclimation in the mist house for 2 months, they were transferred to green house.

Results and Discussion

The internodal and leaf explants were inoculated on MS basal medium with B5 vitamins supplemented with various concentrations and combination of BAP (1.0-3.0 mg/l), NAA (0.5-1.0 mg/l) &2.4-D (0.5- 3.0 mg/l) and IAA (0.5-1.5 mg/l) for culture initiation and callus induction. The internodal explants showed yellow callus induction after 12 days of inoculation. The mean numbers of calli were recorded after 4 weeks of inoculation. In internodal explants showed the best callus induction response on BAP (3.0 mg/l) + NAA (0.0mg/l) + 2.4-D (1.0 mg/l) with mean value of 21/24(87.5) as the best response (Table:1) Plate-I, Fig A and B Similar results has also been suggested by (Farhana maqbool *et al,* 2014) [9] on *Atropaacuminata* in BAP (2 mg/l, 3 mg/l, 5 mg/l) after 17, 34 and 26 days of inoculation. The highest percentage of Callus was recorded from leaf explants on different concentration and combination of plant growth regulators like BAP (3.0mg/l) + NAA (2.0mg/l) with mean of value19/22 (86.3) (Table: 2) Fig. C, D and E. Similar results were obtained for *Atropa accuminata* where BAP (3.0 mg/l) + IAA (2.0 mg/l) and BAP (2.0 mg/l) + IAA (3.0 mg/l). MS medium supplemented with BAP (3 mg/l) gave best results for Callus induction from leaf explants (Farhana Maqbool *et al,* 2014) [9] and (Indrani Chandra *et al,* 2013) [10] PGRs (2, 4-D & BAP) were used for callus induction in *Solanum lycopersicum.* Both auxin concentrations showed callus formation but IBA 1.5 mg/liter concentration showed maximum yield. *Nerium indicum* Mill and *Alangium salviifolium* Linn have been investigated for their primary metabolites (Tanwer *et al,* 2010) [11], (Ayyadurai and Ramar 2016) [5], (Vijayvergia *et al*, 2007) [12]. The *in vitro* callus induction of *Physalis minima* revealed that the tissue culture showed good response in proliferation of in MS medium by supplementing with BAP, NAA, 2. 4-D & IAA. The present study conducted was to establish reliable regeneration protocol for *Physalis minima*, which can be used for easier cultivation, propagation and plant genetic studies. The present investigation has also opened new researcher for genetic manipulation of *Physalis minima* for disease,

Table 1: Callus induction from internodal explants of *Physalis minima* L.

Grouth regulators (mg/l)			Internodal explants [Number of explants responded/ inoculated (%)]	Nature of the callus
BAP	2.4-D	NAA		
1	0.5	-	16 / 24 (66.6)	Yellow fluencies callus YFC
2	0.5	-	18 / 23 (78.2)	Yellow fluencies callus YFC
3	1	-	21 / 24 (87.5)	Yellow fluencies callus YFC
-	1	0.5	17 / 22 (77.2)	Yellow fluencies callus YFC
-	2	0.5	16 / 25 (64.0)	Yellow fluencies callus YFC
-	3	1.0	15 / 23 (65.2)	Yellow fluencies callus YFC
1	-	0.5	14 / 24 (58.3)	Yellow fluencies callus YFC
2	-	0.5	16 / 21 (76.1)	Yellow fluencies callus YFC
3	-	1.0	17 / 23 (73.9)	Yellow fluencies callus YFC

Table 2: Callus inductions from leaf explants of *Physalis minima* L.

Grouth regulators (mg/l)			leaf explants [Number of explants responded/ inoculated (%)]	Nature of the callus
BAP	NAA	IAA		
1	0.5	-	10 / 21 (47.6)	Yellow fluencies callus YFC
2	1.0	-	17 / 24 (70.8)	Yellow fluencies callus YFC
3	2.0	-	19 / 22 (86.3)	Yellow fluencies callus YFC
1	-	0.5	10 / 23 (43.3)	Yellow fluencies callus YFC
2	-	1.0	12 / 21 (57.1)	Yellow fluencies callus YFC
3	-	1.5	16 / 24 (66.6)	Yellow fluencies callus YFC

Plate-I

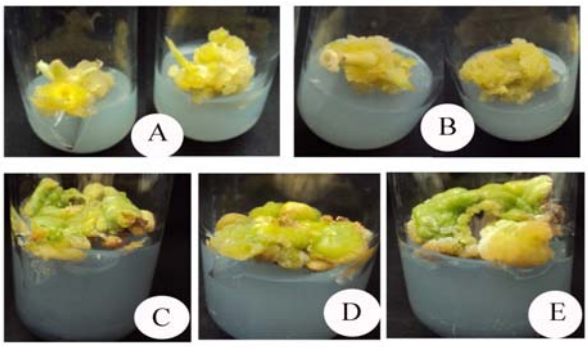

Fig: (A) The callus induction from internodal explants (BAP 2 mg/l +2.4-D 0.5 mg/l)
Fig: (B) The internodal explants produce yellow coloured callus.
Fig: (C) The leaf explants shows green callus induction in the margin of leaf surface.
Fig: (D) Shows the yellowish callus proliferation.
Fig: (E) Shows the green compact Callus.

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